GENETIC POLYMORPHISM IN THE IL-10 PROMOTER

Application Information

The subject matter of this invention was funded under United States Government Contract AR33062 through the National Institutes of Health.

Field of the Invention

The present invention relates generally to a compound and process for identifying polymorphism in an interleukin cytokine promoter, and more particularly, to a compound and process for identifying and typing single nucleotide polymorphisms that code for an interleukin-10 (IL-10) promoter and applying these polymorphisms to delineation of disease susceptibility and severity.

Background of the Invention

Interleukin-10 (IL-10) is known as an anti-inflammatory cytokine, B-cell proliferation factor and is active in autoimmunity, tumorigenesis and transplant tolerance. *Eur. J. Immunogenet.* 1997 24(1):1-8.

IL-10 has pleiotropic effects in immunoregulation and inflammation including enhancing B-cell proliferation and antibody production, and altering macrophage response to infection yet stimulates Fc receptors on the same cells. *Annals Allergy Asthma Immunol.* 1997 79:469-483; *J. Immunol.* 1993 151:1224-1234; and *J. Immunol.* 1992 149:4048-4052. Studies have indicated a role for IL-10 production in the pathogenesis of autoimmune diseases, illustratively including rheumatoid arthritis, systemic lupus erythematosus,

Sjogren's syndrome, inflammatory bowel disease and susceptibility to certain infectious diseases and other functions of the immune system.

Several studies have shown that unaffected family members of SLE patients produce high levels of IL-10 and that first degree relatives of non-survivors of fatal meningococcal disease produce significantly lower levels of IL-10 than relatives of survivors. *Arthritis Rheum.* 1997 40:1429-1435; *Lancet* 1998 351:950-953; and *Lancet* 1997 349:170-173. The implication of a heritable genetic basis for IL-10 production is supported by the concordance of IL-10 production in monozygotic twins which suggest that genetics could account for up to 75% of IL-10 production. *Lancet* 1997 349:170-173.

IL-10 production varies between individuals and through analysis of families, appears to have an inherited component. The IL-10 promoter is highly polymorphic. Two dinucleotide microsatellites (IL-10R, IL-10G) have been identified in the 4.0 kb 5 untranslated region, and three Single Nucleotide Polymorphisms (SNPs) have been identified in the 1.3 kb 5 UTR. *Cytokine* 1995 7:1-7; and *Immunogenetics* 1997 46:120-128. Recent studies have shown that the IL-10.R3 allele of the IL-10.R microsatellite associates with lower IL-10 production while the IL-10.R2 allele, and the IL-10.G14 allele of the IL-10G microsatellite associate with high IL-10 production. *Immunogenetics* 1996 42:444-445; and *Immunogenetics* 1996 45:82-83. In addition, associations have been observed between the GCC haplotype of the three known SNPs (-1082G/A, -819C/T, -592C/A) and high IL-10 production and

Ro antibody production. *Kidney Int.* 1999 56(1):281-8; *Arthritis Rheum.* 1998 41(6):1090-5.

Summary of the Invention

The present invention is a package and process for correlating the properties of IL-10 production and cellular basis for susceptibility to a disease by identifying an IL-10 promoter of a cell and quantifying IL-10 production. Thereafter, IL-10 production by the cell by a second cell expressing a second IL-10 promoter genotype is compared.

The present invention uses a single nucleotide polymorphism or combinations thereof within the IL-10 promoter genotype to identify individual susceptibility to a disease.

A process of the present invention also extends to correlating the IL-10 production of a cell and cellular susceptibility to a disease through identifying IL-10 promoter haplotype and IL-10 production by the cell. Thereafter, IL-10 production by the cell is compared to a second IL-10 promoter haplotype. In particular, single nucleotide polymorphisms are responsible for phenotypical differences in IL-10 production.

The present invention further includes a commercial packaging including reagents for identifying single nucleotide polymorphisms in the IL-10 promoter phenotype of an individual as a test to identify individual susceptibility to a disease associated with IL-10 production. The reagents further include instructions for the use thereof.

Brief Description of the Drawings

Figure 1 shows a schematic representation of the IL-10 promoter with seven novel SNPs found in Caucasian donors. Within this region an additional C/T SNP at -1466 was identified in African Americans. A second SNP at position -429 (G/T) was also found in African American donors. *n=48; **n=14.

Figure 2 shows identification of SNP haplotypes in the IL-10 promoter. The TGA haplotype (*) was identified unambiguously in the independent populations of 128 normal Caucasian and 64 normal African American donors with an approximate haplotype frequency of 0.02 and 0.08, respectively. The TAC haplotype (**) was identified unambiguously in normal African American donors with an approximate haplotype frequency of 0.02.

Figure 3 is a graph showing association of -3575A/-2763A SNP haplotype with production of IL-10 by donors, segregated by distal SNP haplotypes. Homozygosity for haplotypes with an A allele at both -3575 and -2763 was strongly associated with low IL-10 production (p=0.007).

Detailed Description of the Invention

The present invention is based on a novel form of polymorphism in the IL-10 promoter. A novel polymorphism is exploited in a testing methodology which allows for early identification of individuals susceptible to diseases associated with IL-10 function, offering the possibility of early and aggressive treatment in those patients.

The present invention relates to a compound and process for identifying single nucleotide polymorphisms in the -1.2 kb to -4.0 kb region of IL-10 promoter. Preliminary data using the 1.3 kb IL-10 promoter in reporter gene constructs failed to show an association between IL-10 production and the polymorphic sites suggesting that other promoter sites may be involved in IL-10 production. To identify additional SNPs that may play a role in IL-10 production, the present invention sequenced a 4 kb PCR-amplified region of the IL-10 promoter from several individuals phenotyped for high or low IL-10 production. At least seven additional SNPs exist in the -1.2 kb to 4.0 kb IL-10 promoter region. It is appreciated that other techniques known to the art may also be employed, these illustratively include the use of allele specific oligonucleotides as hybridization probes and/or as primers for DNA amplification.

Because of the important role of IL-10 in the immune response, the present invention has utility as a diagnostic to identify high risk patients that warrant early and aggressive treatment. As a diagnostic for infectious disease, the present invention has utility in predicting susceptibility to specific microbes and thereby guiding the use of therapeutics. As a diagnostic for autoimmune disease, the present invention has utility in diseases illustratively including SLE, transplant rejection, Felty's syndrome, allergies, asthma, myasthenia gravis, systemic vasculitis, glomerulonephritides, Sjogren=s syndrome and inflammatory bowel disease. Identification of the appropriate allelic forms further allows for gene therapy transduction of host cells to correct hereditary

limitations in the host's IL-10 promoter genes through delivery of a translatable promoter gene to a host cell. Generally, a predominant "normal" gene of the total human population or a derivative thereof would be delivered.

The present invention provides a method for identifying the IL-10 promoter single nucleotide allelic pattern in human patients which involves testing DNA from individual patients for the presence of different allelic variants. The present invention also encompasses the identification analysis of new single nucleotide allelic forms of the IL-10 promoter, the analysis being achieved using methods well known in the art, such as direct DNA sequencing; single strand conformational polymorphism analysis (SSCP); "HOT" cleavage; denaturing gradient gel electrophoresis (DVGE) and combinations thereof.

Once a new polymorphism has been identified, molecular biological tests are used to haplotype patients for the presence or absence of a given single nucleotide polymorphism. For example, allele specific oligonucleotides may be designed for use as probes and/or as primers in hybridization or PCR based detection methods, respectively.

To facilitate heterozygote detection, a dye primer strategy is used for fluorescence-based automated cycle sequencing of PCR products on an ABI 377. All sequencing primers are designed with an 18bp M13 sequence tag (ABI PRISMJ Dye Primer Cycle Sequencing -21M13 FS and M13REV FS Ready Reaction Kits (ABI, Foster City, CA)). The PCR products are purified with the QIAquick Gel Extraction Kit (Chatsworth, CA). The BigDye terminator sequencing reaction chemistry (ABI) is also used to detect

heterozygosity in IL-10 receptors. This chemistry has the advantage of allowing longer sequence reads and does not require the incorporation of additional M13-based sequence in the primers. It is appreciated that all potentially polymorphic residues must ultimately be confirmed by sequencing in both the forward and reverse directions using the dye-primer based strategy.

Through the establishment of statistically significant correlations between the different single nucleotide polymorphic allelic forms of IL-10 promoter within the -1.2 kb to -4.0 kb region and various physiological or clinical manifestations of IL-10 production levels. These correlations are utilized to provide diagnostic utilities of the present invention. In practicing the present invention, preferably the correlations sought are those between particular IL-10 promoter single nucleotide allelic polymorphs and the IL-10 response to any of the illustratively aforementioned diseases.

The term "allele" or "allelic form" is intended to mean an alternative version of a gene encoding the same functional protein but containing differences in nucleotide sequence relative to another version of the same gene.

The term "allelic polymorphism" or "allelic variant" is intended to mean a variation in the nucleotide sequence within a gene, wherein different individuals in a general population express different variants of the gene.

The term "allelic pattern" is intended to mean the identity of each of the two copies of a particular gene in a patient i.e., homozygosity or heterozygosity.

The term "allelic pattern" is used herein interchangeably with "genotype."

The term "genotyping" as used herein as being the process of determining the allelic patterns of a human individual.

Examination of the DNA sequence encoding the IL-10 promoter by direct sequence analysis shows two sites (-3573 and -2763) associated with IL-10 production. In 52 normal healthy donors phenotyped for high or low IL-10 production in response to an LPS stimulus, the A allele at nt -3575 ($p<0.001$), and the A allele at nt -2763 ($p<0.01$) are associated with low IL-10 production.

Further, the A allele at nt –2849 shows a correlation in donors phenotyped for low IL-10, as summarized in Table 1. Examination of the -3575, -2849 and -2763 haplotypes in 48 SLE patients is compared to that for the group of 52 normal donors in Table 2 in reference to high or low IL-10 production levels in the normal donors.

DNA was obtained from a donor and the presence of DNA sequences corresponding to a particular IL-10 promoter single nucleotide allelic polymorphism are determined. The DNA may be obtained from any cell source or body fluid containing intact nucleic acid bearing cells (expressing a complement receptor). DNA is extracted from the cell source using any of the numerous methods that are standard in the art. Once extracted, the DNA may be employed in the present invention without further manipulation. Direct sequencing is accomplished by enzymatic sequencing by way of methods such as the Sanger method. *Proc. Natl. Acad. of Sci., USA* 1977 74:5463. It is

appreciated that alternate sequencing methods such as chemical sequencing, using the Maxam-Gilbert method. *Proc. Natl. Acad. of Sci., USA* 1977 74:560.

To identify SNPs in the more distal promoter region that might correlate with IL-10 production, we PCR amplified a 4.1 kb DNA fragment from the IL-10 promoter which included the 5'UTR and 43 bases of the ORF. Eight low and six high IL-10 producing Caucasian donors were selected and the promoter region between -1.3 kb and -4 kb sequenced. DNA sequences obtained using the BigDye Terminator Cycle Sequencing system were confirmed by sequencing the reverse strand using the M13-based Dye Primer system. Seven novel genetic SNPs were identified within the -1.3 kb to -4 kb promoter region of IL-10 as per Figure 1 and the frequency characterized in two independent populations. At three of these sites -3575T/A, -2849G/A, -2763C/A both alleles are commonly represented in the populations studied. At each of the other four sites -3715 **A**/T, -2776 **A**/G, -2100**C**/A, -2050**G**/A one allele (in bold) predominates while the other rare allele has a frequency less than 5%.

Sequencing data from the region between -1.3 kb and -4 kb also showed several single base differences compared to published IL-10 promoter sequences (Genbank entries U16720 and X78437), and they confirm the presence of six nucleotides (GCTCAA) at -1469, and two nucleotides (GA) at -1685. Several other nucleotides present in one or the other of the Genbank entries were not present in any of our sequences. Except for differences at the

SNP sites, the 14 Caucasian donor sequences were all identical, suggesting that differences between the Genbank sequences and our data are unlikely to be the result of PCR-induced mutations. To address the possibility that differences in the ethnicity of the donors could explain sequence differences seen in the IL-10 promoters, PCR-amplification and sequencing of the 4 kb IL-10 promoter region was conducted from 14 African American normal donors. In our African American cohort, two novel SNP sites were identified that were not polymorphic in any of the 52 Caucasian normals phenotyped for IL-10 production, Figure 1.

The proximal SNPs in the IL-10 promoter (-1082G/A, -819C/T, -591C/A) form three haplotypes in Caucasians (GCC, ACC and ATA), and a fourth haplotype (GTA) has been reported in southern Chinese (29). To determine the distal IL-10 promoter SNP haplotypes for the three sites with common polymorphisms, the first cohort of 52 Caucasian donors was genotyped. Four haplotypes were observed in donors homozygous at all three sites or heterozygous at only one site. The 4 kb promoter from 19 donors heterozygous at more than one of the common distal SNP sites was then cloned. PCR amplified promoter products were cloned into pGEM-T vector and ten randomly chosen clones from each donor sequenced. Of the 8 possible distal promoter SNP haplotypes, haplotypes in the first group of 52 Caucasian normals were identified, Figure 2. In this cohort, three haplotypes comprised of the common distal SNPs (TGC, AAA and AGA), were present with the highest frequency (0.51, 0.26 and 0.13 respectively) while the other haplotypes,

TAA, AAC and AGC, were present with a low frequency (0.02, 0.05 and 0.04 respectively). In the second, independent Caucasian cohort and in the African American populations (see below), the other two possible distal SNP haplotypes (TGA and TAC) were unambiguously deduced, Figure 2. Therefore, all 8 haplotypes for the three distal sites with common alleles (-3575T/A, -2849G/A, -2763C/A) exist in normal populations.

The genotype distribution in the normal phenotyped Caucasian donors was examined to determine whether any haplotypes were associated with IL-10 production. Table 2 shows that among the three most common haplotypes, both AAA and AGA were each more frequently found in the low compared to the high IL-10 producers. More importantly, donors with two A-[G/A]-A haplotypes were ten-fold more frequent among the 26 low compared to the 26 high producers (42% vs 4%; $p < 0.007$) as shown in Figure 3. Among donors in the lower half of IL-10 production, which had a median IL-10 production of 543 pg/ml, an A-[G/A]-A haplotype was more frequent than among those in the upper half which had a median production of 903 pg/ml (73% vs 35%, $p = 0.012$). The occurrence of two A-[G/A]-A haplotypes was also more common in the lower than in the upper half. Not surprisingly, the TGC haplotype was significantly associated with high IL-10 production ($p < 0.05$)

Extended haplotypes were constructed based on analysis of genotypes in homozygous donors or donors with a single heterozygous site and on analysis of direct sequence information of cloned promoter products. Nine extended haplotypes were identified that include both the proximal and the

distal IL-10 promoter SNPs as shown in Figure 2. Within these extended haplotypes, the TGC distal haplotype is linked to all three proximal haplotypes. However, among the 104 DNA strands analyzed, both the AAA and AGA distal haplotypes was found only with the AGCC proximal haplotype. Conversely, the GATA proximal haplotype was found exclusively with the TGC distal haplotype.

As predicted from the distributions of the AAA and the AGA haplotypes and the absolute linkage of AGCC with these haplotypes in our population, the AAA-AGCC and AGA-AGCC haplotypes predominated in the low producer group (54% vs 23% in high producers, $p < 0.003$). A distribution analysis of the AGCC proximal haplotype alone also showed a higher frequency in the low producer group (low: 65%, high: 44%,) but this distribution did not show as marked a difference between low and high IL-10 producers because of the TGC-AGCC, TAA-AGCC and AAC-AGCC haplotypes, most of which were found in the high producer group. Indeed, if the proximal AGCC were predominantly responsible for the IL-10 production phenotype, then the distribution of distal haplotypes associated with AGCC should be the same in both high and low producers. Table 3 shows that, with AGCC held constant, the distal haplotypes do not occur with equal frequency in both high and low producers. The A-[G/A]-A distal haplotypes are much more frequently found in the low producers (82% vs 52%: $p = 0.032$).

Conversely, with the distal TGC held constant, there was no difference in the distribution of the TGC-GACC, TGC-GATA and TGC-AGCC haplotypes between high and low producers.

To determine if genetically determined capacity for IL-10 production might constitute a susceptibility factor for SLE, the distal SNP haplotypes of 24 SLE patients were examined and compared the distribution of these haplotypes to our high and low IL-10 producing normals. The distribution of SLE patient haplotypes were significantly different from low production normals ($p = 0.001$) and essentially the same as the high producers as shown in Table 4.

While both the G and A alleles at -2849 were equally represented in individuals producing high and low amounts of IL-10, there was a significant over-representation in the frequency of the A allele at both -3575 ($p = 0.02$) and at -2763 ($p = 0.009$) when analyzed individually in low producers compared to high producers as shown in Table 5.

Therefore, the distribution of genotypes at -3575, -2849 and -2763 in 60 African American SLE patients and 64 healthy ethnically matched controls were examined. Table 6 shows that while there was no significant difference in the genotype distribution at the first two sites, there was a significant difference between normals and SLE patients at -2763 ($p < 0.05$). As with our normals producing high levels of IL-10, the -2763A allele frequency was lower and there were significantly fewer -2763A homozygotes in SLE patients versus ethnically matched controls (5 in SLE, 16 in normals, $p = 0.026$).

Inspection of the normal African American population at this site revealed a very high frequency of TT homozygosity and low frequency of AA homozygosity compared to Caucasian normals. Indeed, the -3575T allele frequency in the 64 African American normals was significantly different not only from our Caucasian IL-10 low normals (0.84 vs 0.40) but also from our Caucasian IL-10 high producers (0.84 vs 0.65; $p < 0.00001$ and $p < 0.013$, respectively). To determine if this might be related to the selection of these donors on the basis of IL-10 phenotype, a second population of 128 randomly selected Caucasians was genotyped. The genotype distributions at positions -3575 and -2763 between African Americans and Caucasians were significantly different ($p < 0.00002$ and $p < 0.03$, respectively; Table 7). In particular, the -3575T allele frequency was significantly higher in the African American normals (0.84 vs 0.69; $p < 0.0001$). This enrichment for -3575T and under-representation of -3575A, a genotype associated with high IL-10 production, in African American normals compared to Caucasians suggests that heritable differences in IL-10 production capacity may represent one of the risk factors for the increased prevalence of lupus in African Americans.

The present invention is further illustrated by the following non-limiting scope examples.

Example 1. Normal donor and patient populations:

Fifty two normal Caucasian donors, characterized for quantitative IL-10 production (*J. Immunol. Methods* 1998 218:63-71), provided genomic DNA for genotyping. An additional 128 normal Caucasian donors from the Leiden

University Medical Center blood donor service provided genomic DNA to confirm genotype frequencies. Sixty-four African American normals and 60 African American SLE patients from the University of Alabama at Birmingham also provided DNA for analysis. All SLE patient donors met the revised American College of Rheumatology criteria for systemic lupus (*Arthritis Rheum.* 1982, 25:1271-1277).

Example 2. IL-10 production:

Determination of IL-10 production in lipopolysaccharide-stimulated whole blood assays is described elsewhere. *J. Immunol. Methods* 1998 218:63-71. Briefly, whole blood samples were diluted 1:1 with RPMI 1640 (Gibco Life Technologies, Paisley, UK). LPS (*Escherichia coli* 0111; B4, Boivin method, Difco Laboratories, Detroit, MI) was added to a final concentration of 1 μg/ml and cells stimulated for 24 hours at 37°C under 5% CO_2 atmosphere. Determination of IL-10 concentrations by ELISA (BioSource, Fleurus, Belgium) was performed according to manufacturer's guidelines.

Example 3. PCR amplification and sequencing:

DNA was extracted from whole blood using standard molecular techniques. Forward (F) and reverse (R) primers used to PCR-amplify and sequence the 4 kb region of the IL-10 promoter are given in Table 1. Primers were purchased from Gibco/BRL or from the Oligonucleotide Synthesis Core Facility at The University of Alabama at Birmingham. PCR amplifications were done using the Expand Long Template PCR System according to the manufacturers suggestions (Roche Diagnostic Corp., Indianapolis, IN), or with

recombinant Taq polymerase and deoxynucleoside triphosphates from Gibco/BRL (Grand Island, NY). IL10UPP primer TCCATAGGTCACACAG CAGGCATCCA (SEQ. ID. No. 1) and IL10LWR reverse primer CAGTCAGGAGGACCAGGCAACACAGC (SEQ. ID. No. 2) were used to PCR amplify a 4.1 kb fragment of the IL-10 gene containing 4.057 kb of the 5'UTR and 43 bases of the ORF. Typically, 125 ng of genomic DNA template were used in a 50 μL PCR reaction performed in a Perkin Elmer 9600 Thermal Cycler.

For genotyping, IL-10UPP primer (SEQ. ID. No. 1) and IL10S9R reverse primer TTTGAGACAGAGTCTCGCTCTG (SEQ. ID. No. 3) were used to amplify a 1.443 kb IL-10 promoter fragment containing the -3575 and -2763 polymorphic sites. A typical 50 μL PCR reaction contained 1x PCR buffer, 1.5 mM $MgCl_2$, 0.2 M dNTPs, 0.2 mM primers and 2.0 U Taq polymerase. PCR amplified products were electrophoresed in a 2% agarose gel and DNA fragments purified from excised gel slices using the QIAquick Gel Extraction Kit (Qiagen Inc., Chatsworth, CA). DNA sequencing was performed using 10 ng of gel-purified DNA and the ABI PRISM BigDye Terminator and Dye Primer Cycle Sequencing Ready Reaction Kits (PE Applied Biosystems, Foster City, CA) followed by electrophoresis on the ABI 377 automated sequencer. To facilitate confirmation of heterozygotes, M13-labeled primers and M13-based sequencing kits were used.

Example 4. Cloning of IL-10 promoter segments:

For extended SNP haplotype determination the 4.1 kb IL-10 promoter fragments were cloned into pGEM-T vector (Promega, Madison, WI). Plasmids were propagated in *E. coli* DH5α and plasmid DNA purified from bacterial cultures using the QIAprep Spin Miniprep Kit (Qiagen).

All DNA sequence comparison alignments were done using DNASTAR SeqMan or MegAlign programs (DNASTAR Inc., Madison, WI). DNA sequence motif searches for putative transcription factor binding sites were done using TESS, MatInspector V2.2 and TFSEARCH web-based search programs.

Example 5. Statistical Analysis:

The Chi-square test was used to compare differences in the distributions of phenotypes and allele frequencies. A p value of 0.05 was used to reject the null hypothesis.

Any patents, applications or publications mentioned in the specification are indicative of the levels of those skilled in the art to which the invention pertains. These patents and publications are herein incorporated by reference to the same extent as if each individual publication or patent was specifically and individually indicated to be incorporated by reference.

Table 1

SNPs in –4 kb to –2 kb portion of IL-10 promoter

	-3575			-2849			-2763		
	TT	TA	AA	GG	GA	AA	CC	CA	AA
High IL-10	9	16	1	11	15	0	13	12	1
Low IL-10	8	5	13	10	12	4	8	8	10

Table 2

Haplotypes for SLE patients and healthy donors producing high and low IL-10 levels

	Haplotypes			SLE vs High IL-10		SLE vs Low IL-10	
	-3575	-2849	-2763	SLE	High	SLE	Low
1.	T----------	G/A----------	C	28	33	28	19
2.	T----------	G/A----------	A	6	1	6	0
3.	A----------	G/A----------	C	5	4	5	5
4.	A----------	G/A----------	A	9	14	9	28
					p=N/S		p=0.003

Table 3

Distribution of AGCC-Linked Distal Haplotypes in High and Low IL-10 Producers

Extended Haplotype	Phenotype	
Distal — Proximal	Low	High
TGC — AGCC	3	4
TAA — AGCC	0	2
AAA — AGCC	18	9
AGA — AGCC	10	3
AAC — AGCC	1	4
AGC — AGCC	2	1

A-[G/A]-A-A-G-C-C vs others: p=0.032, 2 x 2 chi-square analysis

Table 4

Distribution of IL-10 Haplotypes in SLE Patients

Haplotypes			SLE vs Low IL-10*		SLE vs High IL-10	
-3575	-2849	-2763	SLE	Low	SLE	High
T	G/A	C	28	19	28	33
T	G/A	A	6	0	6	1
A	G/A	C	5	5	5	4
A	G/A	A	9	28	9	14

*p = 0.001, 4 x 2 chi-square analysis

Table 5

Association of -3575 and -2763 SNP Alleles with IL-10 Phenotypes

	-3575		-2849		-2763	
	T	A	G	A	C	A
High IL-10	34	18	37	15	38	14
Low IL-10	21	31	32	20	24	28

-3575, p = 0.02; -2763, p = 0.009; 2 x 2 chi square analysis.

Table 6

Distribution of IL-10 Distal Promoter SNP Genotypes in African American SLE Patients

	-3575			-2849			-2763		
	TT	TA	AA	GG	GA	AA	CC	CA	AA
AA SLE	40	19	1	38	19	3	28	27	5
AA Normals	47	12	5	36	22	6	25	23	16

-2763, $p < 0.05$, 2 x 3 chi-square analysis.

Table 7

Distribution of IL-10 Distal Promoter SNP
Alleles in African Americans and Dutch Caucasians

	-3575			-2849			-2763		
	TT	TA	AA	GG	GA	AA	CC	CA	AA
Dutch Normals (N = 128)	51	64	13	71	49	8	51	63	14
AA Normals (n = 64)	48	11	5	36	22	6	25	23	16

-3573, $p < 0.00002$; -2763, $p < 0.03$; 2 x 3 chi-square analysis.